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Tuber xanthomonosporum, a new *Paradoxa*-like species from China

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Abstract — A new species, *Tuber xanthomonosporum*, is described based on specimens collected under *Pinus yunnanensis* in Panzhihua, Sichuan Province, China. *Tuber xanthomonosporum* invariably has only one spore per ascus. It can be distinguished from the three other Chinese *Paradoxa*-like species — *T. gigantosporum*, *T. sinomonosporum*, and *T. glabrum* — by its whitish to yellow-brownish gleba, two-layered peridium, and spiky cystidia. Molecular analysis also supports *T. xanthomonosporum* as a unique species.

Key words — taxonomy, *Pezizales*, Yunnan

Introduction

The genus *Paradoxa* was erected by Mattirolo in 1935 in the *Tuberaceae* to accommodate *Paradoxa monospora* Mattir., a species which, invariably, has one-spored asci (Montecchi & Sarasini 2000; Læssøe & Hansen 2007). For the following 50 years the genus remained monotypic until Wang & Hu (2008) transferred *Tuber gigantosporum* into *Paradoxa.* Wang & Li (1991) originally described *T. gigantosporum* based on a single specimen collected from Huidong County, Sichuan, China, in 1988. The species was characterized by its extremely large elliptical ascospores and one-spored asci, a feature that was eventually recognized as being important when a second *T. gigantosporum* collection was made in Yongren County, Yunnan, China, in 2002. Since then three other *Paradoxa*-like species have been reported: *Tuber* sp. 1 (Kinoshita et al. 2011), *Paradoxa sinensis* (Fan et al. 2012), and *Tuber glabrum* (Fan et al. 2014). Recent molecular phylogenetic analysis of truffle species supported *Paradoxa* within *Tuber* (Kinoshita et al. 2011, Fan et al. 2013), and subsequently Fan et al. (2014) transferred *P. sinensis* into *Tuber* with the replacement name *T. sinomonosporum*.

In 2012 during a survey in Panzhihua in the southwestern corner of Sichuan, China, we found truffles that invariably had one-spored asci. Here we describe this *Paradoxa*-like truffle as a new species of *Tuber* and briefly review the molecular relationships of truffle species with single-spored asci.

TABLE 1: Origin of the fungal ITS sequences. (New sequences are indicated in bold font.)

TAXON	VOUCHER OR CODE	GEOGRAPHICAL ORIGIN	GENBANK NO.
Choiromyces meandriformis	isolate RH691	USA	HM485330
	isolate GB285	USA	HM485331
Gymnohydnotrya australiana	isolate OSC130601	USA	JQ925629
Tuber sp. 1	isolate K201	Japan	AB553344
Tuber sp. 2	isolate K215	Japan	AB553356
T. borchii	clone Bo6	Italy	AF106890
	strain Z43	Italy	AF250291
	Unknown	France	AF132505
T. canaliculatum	JT12670	USA	GQ221455
	JT23942	USA	GQ221456
T. excavatum	TE-M	Poland	KC330228
	isolate zb3281_215	Germany	HM152020
	TE-WD	Poland	KC330227
T. foetidum	isolate B-2452	Hungary	AJ557543
	isolate B-2489	Hungary	AJ557544
T. fulgens	isolate y466	Germany	HM152014
	isolate zb3386_202	Germany	HM152024
	isolate M2435	USA	HM485358
T. glabrum		China	KF002731
		China	KF002732
T. indicum	strain T39	China	JQ638997
	strain T47	China	JQ639005
	strain T49	China	JQ639007
T. latisporum	HKAS 44315	China	DQ898183
	HKAS 42380	China	DQ898184

T. liui	HKAS 48269	China	DQ898182
T. macrosporum	isolate JT19458	USA	HM485372
	isolate S7510	Germany	JF926121
T. maculatum	clone Mac1	Italy	AF106889
	1967	New Zealand	EU753269
T. melanosporum	strain1015	Israel	AF167096
	LHB-mic-Tmel-Qc-09-1	Spain	GU810153
	LHB-mic-Tmel-Qr-09-1	Spain	GU810152
T. panzhihuanense	strain DXJ260	China	JQ978644
	strain DXJ276	China	JQ978650
T. pseudoexcavatum	isolate CJ408	USA	HM485381
	strain E	China	JQ638958
	strain Q	China	JQ638982
	strain T48	China	JQ639006
T. rufum	1798	Italy	EF362473
	1447	Italy	EF362477
T. sinomonosporum	BJTCFAN150	China	KF002729
T. umbilicatum	HKAS48267	China	GU979032
	HKAS44316	China	GU979031
	isolate T104	China	FJ797879
	isolate T117	China	FJ797880
T. xanthomonosporum	**YAAS L3185**	**China**	**KJ162154**
	YAAS L3186	**China**	**KJ162155**
	YAAS L3187	**China**	**KJ162156**
T. zhongdianense	HKAS 45388B	China	DQ898186
	wang0299	China	DQ898187

Materials & methods

Morphological studies

Specimens of a truffle with asci containing a single spore were collected from Panzhihua, Sichuan, China, in 2012. Specimens are deposited in the Herbarium of Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, China (YAAS).

The samples were examined at the Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, China and Sichuan Panzhihua Academy of Agriculture and Forestry, Panzhihua, Sichuan, China. Macroscopic characters were described from fresh specimens and microscopic features described from dried specimens following the methods of Yang & Zhang (2003). Photographs were made under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto double-sided tape and mounted directly on a SEM stub. They were then coated with gold-palladium and examined and photographed with a JEOL, JMS-5600LV SEM.

DNA amplification & sequence analysis

DNA was extracted using CTAB (Doyle 1987) modified by adding 200 µL 5M potassium acetate after 4 × CTAB treatments. The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS-rDNA region. PCR reaction solution and cycling parameters used by Chen & Liu (2007) were adopted. The amplification products were electrophoresed on a 1% agarose gel and purified with Sangon's purification kit. Sequencing was performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL automatic sequencer.

In order to determine whether the specimens represented a new species, we compared our *Tuber xanthomonosporum* ITS-rDNA sequences with 48 ITS-rDNA sequences downloaded from NCBI. An ITS-rDNA sequence of *Gymnohydnotrya australiana* B.C. Zhang & Minter was also included as outgroup. Sequence alignment and phylogenetic analysis protocols followed Chen & Liu (2007).

Results

Phylogenetic analysis

A total of 52 ITS sequences were included in a phylogenetic analysis (Table 1, Plate 2). Of the 712 total characters analyzed, 248 are constant, 51 parsimony-uninformative, and 413 parsimony-informative (L = 1979, CI = 0.481, RI = 0.812, RC = 0.390). ITS sequence analysis revealed that the three *Paradoxa*-like species (*Tuber* sp. 1, *T. sinomonosporum*, *T. glabrum*) form a well-supported subclade (bootstrap support = 98%) within Clade I, which also included *T. macrosporum*, *T. canaliculatum,* and *Tuber* sp. 2. In contrast, *T. xanthomonosporum* does not cluster with the other *Paradoxa*-like species but forms a new Subclade I in Clade II (with low bootstrap support) alongside Subclade II (/melanosporum) and Subclade III (/rufum).

Taxonomy

Tuber xanthomonosporum Qing & Y. Wang, **sp. nov.** Plate 1

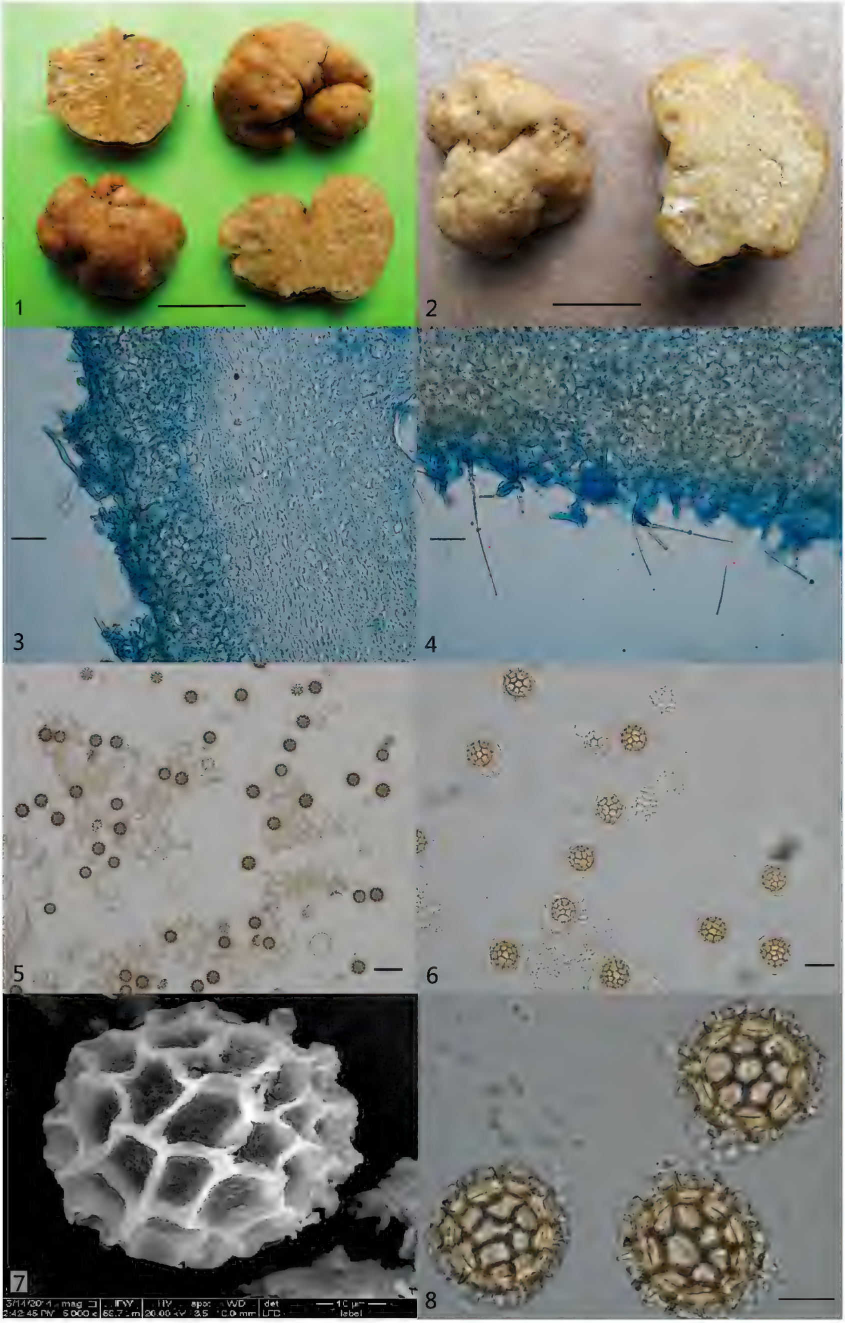
MycoBank MB 807668

Differs from other *Tuber* species with single-spored asci by its whitish to yellow-brownish gleba and two-layered peridium with spiky cystidia.

Type: China, Sichuan Province, Panzhihua, 26°29′N 102°01′E, under *Pinus yunnanensis* Franch., 15 Nov. 2012: Y. Qing Pan1201 (**Holotype**, YAAS L3185; GenBank KJ162154).

Etymology: *xanthomonosporum* from Latin refers to the yellow brownish gleba and single spored asci.

Plate 1. *Tuber xanthomonosporum* (YAAS L3185, holotype): **1**, **2**. Fresh ascomata showing surface features and cut sections. **3**. Cross section of peridium showing two layers of pseudoparenchymatous and interwoven tissue with some cystidia. **4**. Cross section of outer layer of peridium showing two kinds of cystidia: tall and tapered (arrows), and short and obtuse (arrow head). **5–7**. Ascospores and asci (invariably with only one spore). **8**. Ascospores showing ornamentations. Scale bars: 1, 2 = 1 cm; 3, 8 = 20 µm; 4, 7 = 50 µm; 5 = 100 µm; 6 = 40 µm.


1
2
3
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LFD
10 µm
label
8

Ascomata subglobose or irregular and much lobed with deep furrows, firm and a little rubbery, whitish to yellow-brownish, up to 3.0 cm broad, rough with hairs. Odor slightly garlicky when mature. Peridium 160–200 µm thick, composed of two layers; the outer layer complex, 50–70 µm thick, pseudoparenchymatous, composed of irregular globose to rectangular cells, 19–32 × 13–15 µm diam and thin-walled; the outmost cells giving rise at the surface to cystidia of two types; one tall, thin, tapered, reaching 50–90 × 2–4 µm and sometimes with a small teat on the wall, hyaline; the other short, obtuse, 1–2-septate, 15–20 × 4–5 µm; the inner layer 110–130 µm thick, composed of mainly interwoven, colourless hyphae of 2–4 µm diam with some big cells of 2–4 µm diam, that gradually merge into the gleba tissue. Gleba solid, whitish when young, becoming yellow-brownish at maturity, marbled with distinct, white and meandering veins, merging at many points with the peridium. Asci 60 × 70 µm, globose to subglobose, sessile, slightly thick walled 2–3 µm thick, invariably 1-spored and randomly dispersed in glebal tissue. Ascospores globose, 41–43 × 43–46 µm; spore walls 2.5 µm thick, brown at maturity, ornamented with a regular alveolate-reticulum, 2–3 µm deep, constituted of mostly hexagonal meshes 8–15 × (5–)7–13 µm, 4–5 across the spore width.

Ecology & distribution — Hypogeous, solitary or in groups in calcareous soils with pH 6.8–7.6 under *Pinus yunnanensis* and forming ectomycorrhizae with its roots, alt. 1500–2000 m, fruiting from autumn to winter. Known only from Sichuan, China.

Additional specimens examined: **CHINA, Sichuan Province, Panzhihua,** 26°29′N 102°01′E, under *Pinus yunnanensis,* 15 Nov. 2012, C.Y. Liu Pan-No-10 (YAAS L3186; GenBank KJ162155); C.Y. Liu Pan-No-40 (YAAS L3187; GenBank KJ162156).

Discussion

The new *Tuber* species, *T. xanthomonosporum* from Sichuan, produces only one spore per ascus, the diagnostic character of the now defunct genus, *Paradoxa* (Mattirolo 1935; Fan et al. 2014). Recent phylogenetic studies of *Paradoxa*-like species (Kinoshita et al. 2011; Fan et al. 2014) found that they group with *Tuber* species. Our phylogenetic analysis groups *T. xanthomonosporum* with *Tuber* species but not with other *Paradoxa*-like species, despite sharing the common character of having only one spore per ascus. Our work therefore supports the conclusion by Fan et al. (2014) that *Paradoxa*-like truffle species do not form a monophyletic group. While *Tuber sinomonosporum* [≡ *P. sinensis*], *T. glabrum,* and *T.* sp. 1 (the Japanese *Paradoxa*-like new species) do group together, they also group with *T. macrosporum* and *T. canaliculatum* in Clade I (/macrosporum). These species in Clade I share the same morphological feature of having red-brown gleba. *Tuber xanthomonosporum* groups in Clade II with the /melanosporum and /rufum subclades but with very low bootstrap support (<50%). Morphologically, *T. xanthomonosporum* differs greatly from

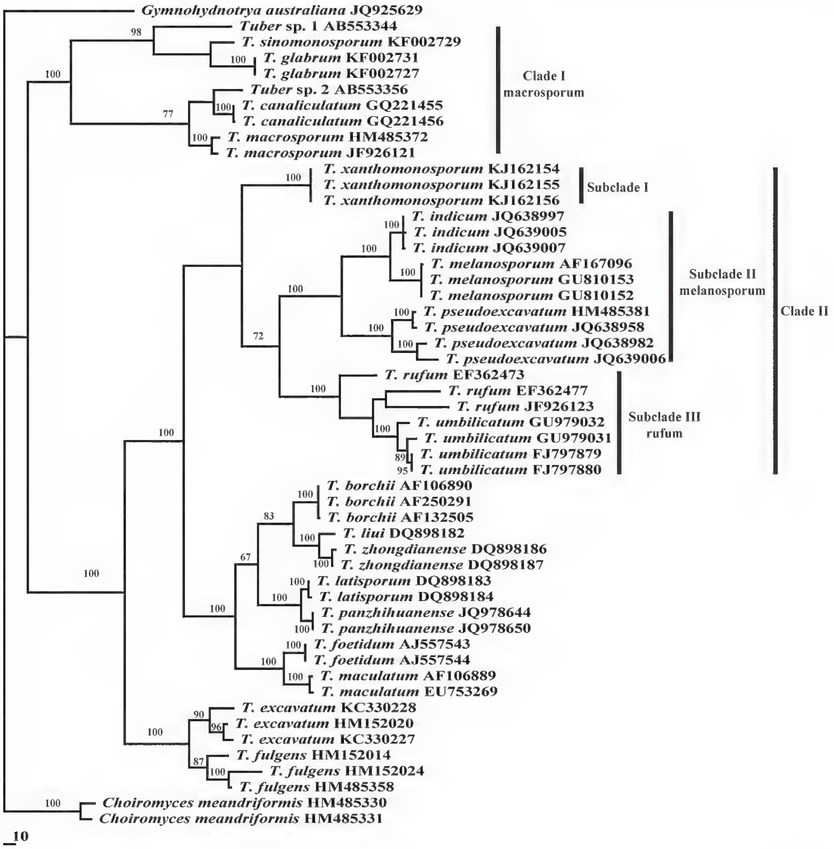

PLATE 2. One of eight most parsimonious trees constructed with ITS sequences of *Tuber xanthomonosporum* and related species. MP Bootstrap values greater than 50% are indicated at nodes.

the species in these two subclades in its whitish to yellow-brownish gleba and reticulate spores. Given the few species and low support for the species now placed in Clade II, the phylogenetic relationships need further study with more samples and gene loci.

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